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Introduction

We have made significant progress in our analysis of genomic alterations, including those of microRNAs, in prostate cancer over the last two years. This work was performed with the support of other funds, but it helped us build a strong foundation for the planned work on microRNAs and gene regulation in prostate cancer under the requested no-cost extension.

As was discussed with the DoD program officers, the funding had not been made available to the new investigator due to an internal procedural error at MSKCC, after the death of the original investigator, William Gerald, M.D. However, our separately funded activities in prostate cancer research, as recently published (see below), have revealed very interesting aspects of the relation between DNA copy number changes, disease prognosis, and, potentially, response to therapy, as well as a first set of microRNA expression profiles. We are therefore in an even better position than two years ago to implement the research program on microRNAs and gene regulation in prostate cancer, as originally envisaged by William Gerald, building on our results so far and exploiting new next generation sequencing technology.

We are grateful for the opportunity to perform this work under the requested no-cost extension.

Body

We had previously described our initial analysis of microRNA expression profiles in 149 human prostate cancer samples. We had found three distinct expression clusters. We have since, supported by other funds, expanded the genomic profiling to a total of 247 prostate cancer samples, and generated DNA copy-number profiles and mRNA expression profiles for most of these. We also sequenced the coding regions of 120 selected genes. The main results of our analysis are (Taylor et al., 2010):

- The androgen receptor co-activator *NCOA2* is amplified in primary and metastatic disease
- TMPRSS2-ERG positive tumors associate with 3p14 loss and its candidate target genes *FOXP1*, *RYBP*, *SHQ1*
- The degree and pattern of CNAs in primary tumors is associated with risk of relapse

The third point regarding CNA is the main finding of our study. Patterns of DNA copy-number alterations are indicative of disease relapse (as defined by biochemical recurrence, i.e. a rise of PSA levels after radical prostatecomy). Patients with no or very few DNA copy-number changes have a very low risk of disease recurrence, and patients with significantly altered genomes have a very high risk (**Fig. 1**). These copy-number changes are a better predictor than Gleason score or other clinical variables, and some of the regions defining the clusters contain microRNAs.

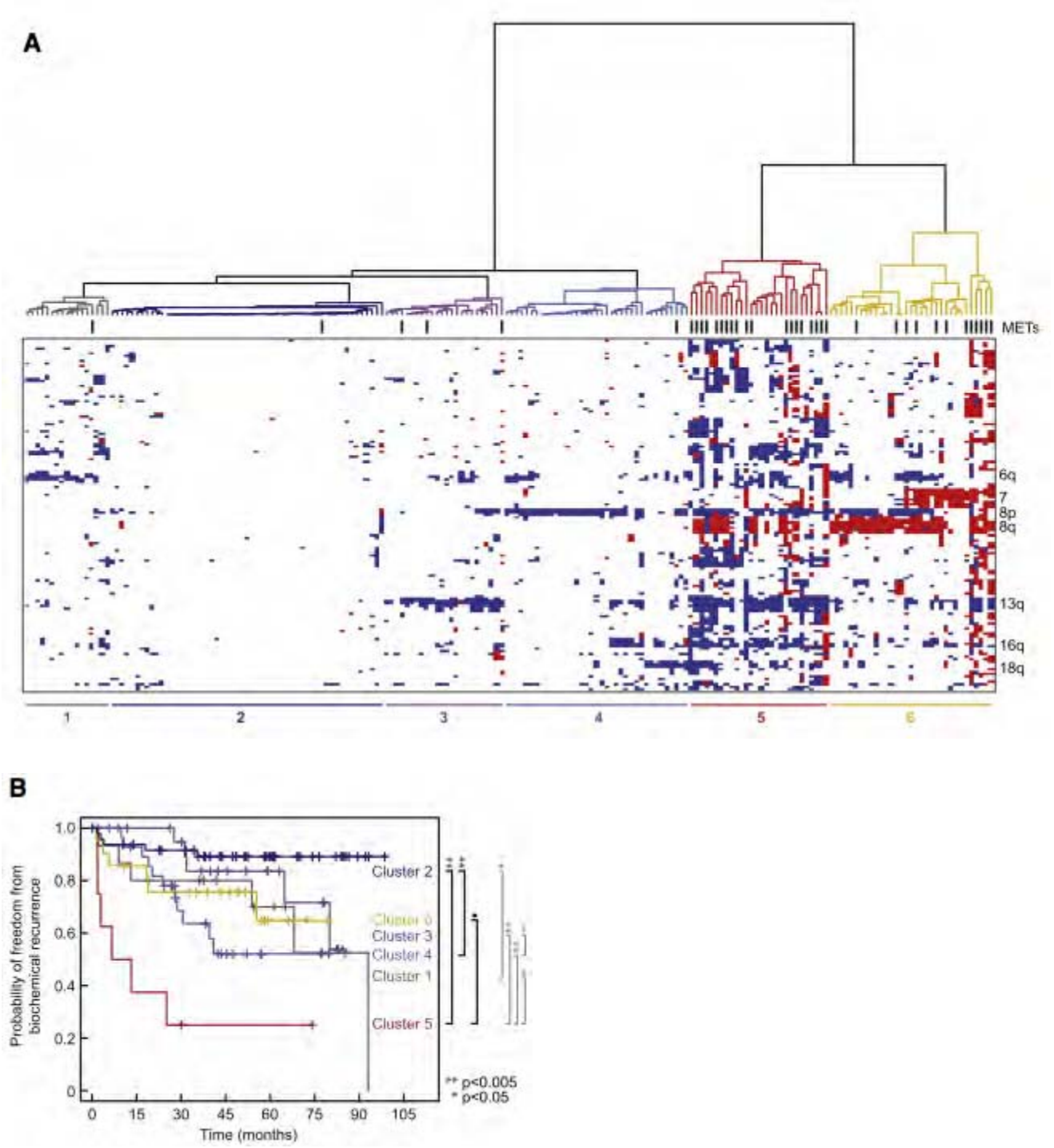

Figure 1. Genomic Aberrations Identify Clinically Distinct Subtypes of Prostate Cancer. (A) *Unsupervised hierarchical clustering of copy-number alterations reveal six distinct groups of prostate cancer samples (heat map: red - amplification, white - copy-neutral, blue - deletion). These are ordered on the basis of their group membership (dendrogram, groups in different colors; metastatic samples - hashes). Selected genomic regions are indicative of group membership (labels on right).* ***(B)*** *The different prostate cancer subtypes, especially cluster 2 and cluster 5, differ significantly in the risk of biochemical recurrence (p-values from a log-rank test relative to randomized cluster assignments).*

All data from this study, including the microRNA expression data, are available through the cBio Cancer Genomics Portal (**http://www.cbioportal.org/**) at MSKCC. This portal provides simple and convenient

visualization, analysis and download of cancer genomics data, and is an important tool in our day-to-day analysis (through our R package and the Portal's WebAPI).

While global expression profiles of microRNAs in prostate cancer are not indicative of disease outcome, individual microRNAs may still be important for disease progression, as documented in other tumor types (see, e.g., our recent results on mir-143 as a determinant of aggressive liposarcoma, our work and Sam Singer, private communication). We are currently pursuing several tracks of investigation to identify single microRNAs or groups of microRNAs characteristic of disease outcome.

1. MicroRNAs affected by DNA copy-number changes in prostate cancer
DNA copy-number analysis using RAE (Taylor et al., 2008) identified 30 recurrent amplifications and 36 recurrent deletions in prostate cancer. Ten (Table 1) and five (Table 2) of these regions, respectively, contain microRNAs. Although none of these microRNAs appear to be focally targeted in these regions, or show striking patterns of expression changes that can directly be explained by DNA copy-number changes, even smaller changes of microRNA expression may have functional consequences, so we plan to follow up on some of these microRNAs experimentally (see below).

Table 1. Amplifications spanning microRNAs in prostate cancer.

Locus	Region (Mb)	Frequency†	Q-value‡	No. of genes§	Genetic element of interest	microRNAs
1q21.2-q21.3	148.035-151.149	8.2 (1.5)	0.0124	101,1,37	MCL1,ARNT	hsa-mir-554
1q21.3-q22	151.576-153.639	8.2 (1.5)	0.0096	77,3,23	-	hsa-mir-190b,hsa-mir-92b,hsa-mir-555
3q26.1-q26.2	168.467-172.385	8.2 (0)	0.0029	27,2,4	-	hsa-mir-551b,hsa-mir-569
5p15.2-p15.1	8.258-16.048	8.8 (1)	0.0046	15,1,6	CCT5,CTNND2,DNAH5,TRIO,FBXL7	hsa-mir-887
5p13.3-p13.1	32.133-41.178	12.4 (2.6)	5.71E-04	50,2,16	AMACR,RICTOR,SKP2	hsa-mir-579,hsa-mir-580
7q32.1-q33	127.022-133.401	11.9 (1.5)	1.71E-06	47,8,17	-	hsa-mir-593,hsa-mir-129-1,hsa-mir-182,hsa-mir-96,hsa-mir-183,hsa-mir-335,hsa-mir-29a,hsa-mir-29b-1
7q33-q34	133.839-140.236	11.9 (1)	<9.75E-08	43,1,22	-	hsa-mir-490
9q21.33-q32	88.662-115.462	11.9 (1.5)	0.0013	175,7,39	-	hsa-let-7a-1,hsa-let-7f-1,hsa-let-7d,hsa-mir-23b,hsa-mir-27b,hsa-mir-24-1,hsa-mir-32
9q32-q33.1	115.813-118.13	7.2 (0)	0.0238	15,1,0	-	hsa-mir-455
9q33.2-q34.3	124.017-140.242	9.8 (1)	0.0066	271,8,65	-	hsa-mir-600,hsa-mir-601,hsa-mir-181a-2,hsa-mir-181b-2,hsa-mir-199b,hsa-mir-219-2,hsa-mir-126,hsa-mir-602

† Single-copy gain (amplification in parentheses)
‡ False-discovery corrected p-value
§ Number of genes (NCBI), microRNAs, and genes with correlated transcript expression

Table 2. Deletions spanning microRNAs in prostate cancer.

Locus	Region (Mb)	Effective frequency†	Q-value‡	No. of genes§	Genetic element of interest	microRNAs
2q14.3-q22.3	127.205-145.865	24.2 (5.7)	6.82E-05	65,1,26	-	hsa-mir-128-1
6q12-q22.33	69.392-127.561	45.4 (17)	<5.64E-08	213,5,120	MAP3K7,CASP8AP2,RRAGD	hsa-mir-30c-2,hsa-mir-30a,hsa-mir-587,hsa-mir-548b,hsa-mir-588
8p21.3	21.987-23.088	47.9 (4.1)	<5.64E-08	24,1,15	PPP3CC,SORBS3,BIN3,RHOBTB2,TNFRSF10B,TNFRSF10C	hsa-mir-320
8p21.3-p11.21	23.353-42.014	57.2 (21.1)	<5.64E-08	102,1,63	-	hsa-mir-486
12p13.31-p12.3	6.179-17.36	39.7 (21.6)	<5.64E-08	166,4,76	TNFRSF1A,CDKN1B,DUSP16,ETV6	hsa-mir-200c,hsa-mir-141,hsa-mir-613,hsa-mir-614

† heterozygous deletion (homozygous deletion in parentheses)
‡ False-discovery corrected p-value
§ Number of genes (NCBI), microRNAs, and genes with correlated transcript expression

2. MicroRNA target genes and function in prostate cancer

State of the art algorithms for sequence based computational prediction of miRNA targets suffer from very high false-positive ratios (>70%) when evaluated on experimental datasets. It is therefore essential to incorporate additional information to determine miRNA targets *in vivo*. To study the functional consequences of miRNA regulation in prostate cancer, we have been developing methods that integrate the rich miRNA expression and mRNA expression data sets. miRNAs repress gene expression levels by binding to the 3'UTR of the mRNA, and the expectation is therefore that mRNA targets have lower expression levels in tumor samples where miRNA expression is high, and vice-versa. We have developed methods to quantify such reciprocal expression relationships between all pairs of miRNAs and mRNAs across all tumor samples. Because expression of some genes is strongly driven by genomic alterations in the tumors, these genes might be more difficult to probe for reciprocal expression relationships with miRNAs. We are therefore also evaluating models which incorporate and correct for the DNA-copy number levels of target mRNAs when quantifying reciprocal expression relationships between pairs of miRNAs and mRNAs. Coupled with sequence-based miRNA target prediction algorithms, this information will be important to predict targeting and function of individual miRNAs in the tumors.

Using the strongest expression relationships (correlations and anti-correlations) between all pairs of miRNAs and mRNAs in the prostate tumor datasets, we have constructed a functional map for miRNAs in the tumors (Figure 2). This map groups genes based on their miRNA correlation signatures and miRNAs based on their gene correlation signatures. Genes clustered together in this map could potentially be regulated through the same program of miRNAs. By statistically evaluating clustered genes for over-representation in pathways and

biological processes, this analysis also provides information on the potential biological function of both individual and groups of miRNAs. The results for example suggest that:

- Five miRNAs (miR-106b, miR-93, miR-200c, miR-19a, miR-25) could be specifically involved in regulating cell adhesion processes and extra cellular matrix (ECM) receptor interaction.
- Two miRNAs (miR-23a, miR-27a) could be involved in regulating metabolic pathways and endocytosis.

These predictions were tested experimentally in prostate cancer cell lines.

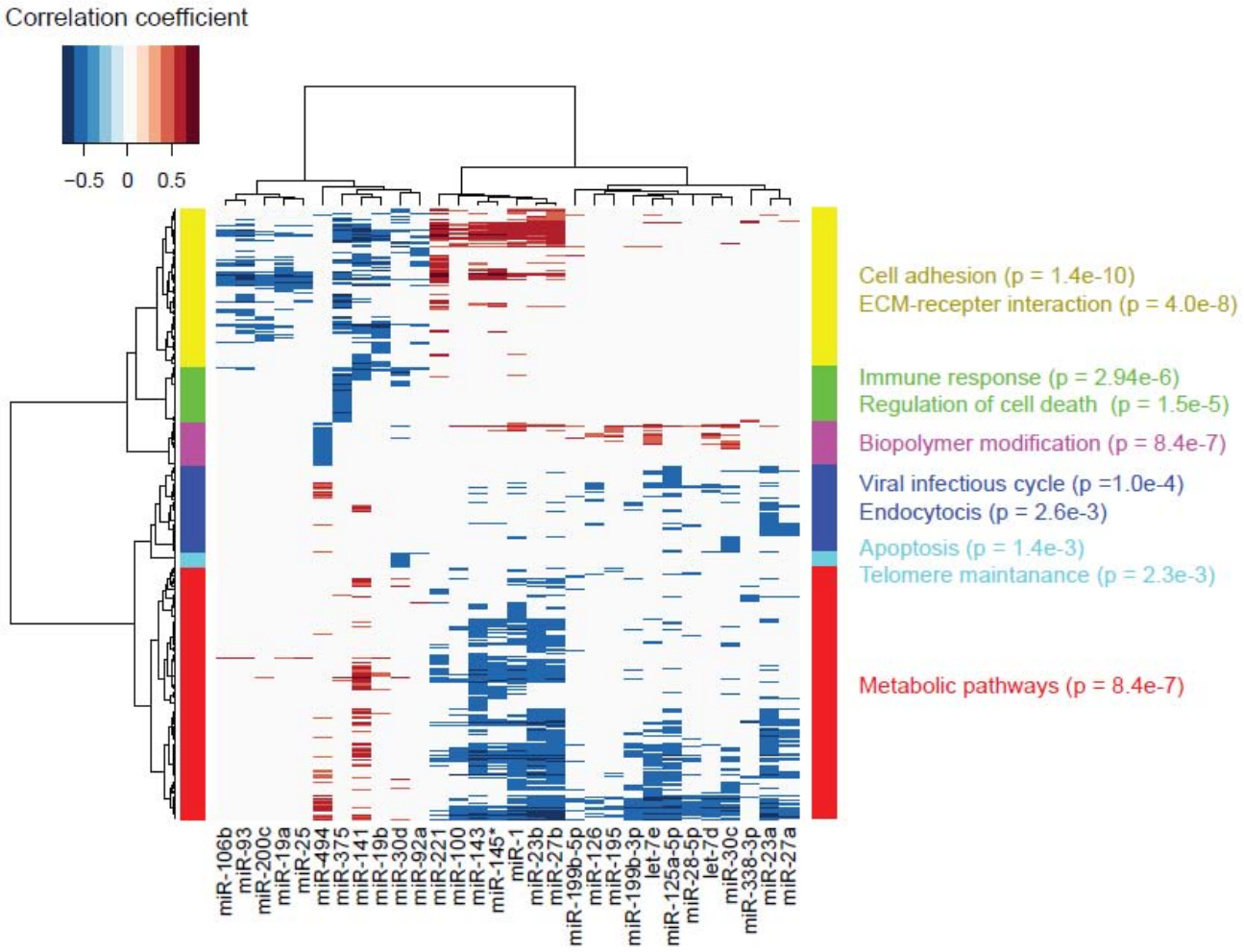

Figure 2. A map of miRNA function in prostate cancer.
The miRNA and mRNA pairs with strongest expression relationships (correlations and anti-correlations) in the prostate cancer data set were selected. Genes (rows) were clustered based on their miRNA correlation signatures, and miRNAs (columns) were clustered based on their gene correlation signatures. The gene clusters were tested for over-representation in pathways and biological processes (the most significant categories are outlined in the right column).

Future work (no-cost extension)

In our continuing genomic analysis of prostate cancer, we are planning the following experiments and analyses under the proposed no-cost extension, in addition to completing the research as outlined in the original proposal (see W.Gerald's grant application on file):

- The most interesting microRNAs (see above) will be investigated experimentally: We plan to transfect microRNA mimics and microRNA inhibitors (antagoMirs) into prostate cancer cell lines (LnCap and VCap) and study their effects on proliferation, apoptosis, and cell migration/invasion.
- We have submitted three triplets of primary prostate cancer, metastatic prostate cancer, and normal prostate from the same patients to Illumina, Inc., for whole genome sequencing. While the sample size is still small, this pilot study will give us, in addition to full genomic data, a first look at mutations in microRNAs and in potential microRNA target sites in different stages of the disease and reference normals.
- Funded by a different grant, we are also starting whole exome sequencing of 50 pairs of primary prostate cancer and matched normal prostate samples (in collaboration with Dick McCombie at Cold Spring Harbor). The sequencing will include the coding regions of all exons, but also all microRNAs, which could uncover novel mutations in microRNAs. This research will leverage the available funding, as exon profiling and microRNA profiling in combination give a refined view of the impact of microRNAs on gene regulation of important disease genes in prostate cancer.

Key Research Accomplishments

- Completed the genomic characterization of almost 250 primary and metastatic prostate cancer samples, which, in addition to microRNA expression profiles, included DNA copy-number profiling, mRNA expression, and exon-sequencing of selected genes.
- Identified microRNAs that are regulated by copy-number changes
- Identified biological processes that appear to be regulated by changes in microRNA expression.

Reportable Outcomes

Manuscript:
Taylor et al. Integrative genomic profiling of human prostate cancer. Cancer Cell (2010) vol. 18 (1) pp. 11-22.

Online database:
http://cbioportal.org/

Conclusion

The work performed so far will serve as a strong foundation for the remaining planned work on microRNAs and gene regulation in prostate cancer under the requested no-cost extension. We expect to complete these work packages and the remaining items under the original grant application within 18 months from award of the no-cost extension. Progress will be summarized in the next scheduled report and in person at the DoD IMPaCT (Innovative Minds in Prostate Cancer) Meeting in Orlando, Florida, in March 2011.

References

Taylor et al. Functional copy-number alterations in cancer. PLoS ONE (2008) vol. 3 (9) pp. e3179.
Taylor et al. Integrative genomic profiling of human prostate cancer. Cancer Cell (2010) vol. 18 (1) pp. 11-22.